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A HYBRIDIZATION PROCESS AND A METHOD FOR DETECTING GENETIC VARIATION EMPLOYING SAME AND AN APPARATUS THEREFOR

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SPECIFICATION

1. Title of Invention

A Hybridization Process and a Method for Detecting Genetic Variation Employing Same and an Apparatus Therefor

2. Claims

1. In the context of a hybridization process for a nucleic acid sample and a nucleic acid probe, a hybridization process for a nucleic acid sample characterized in that a nucleic acid probe is fixed within an electrophoretic carrier and a nucleic acid sample is made to move within the electrical [*probably a typo for* "electrophoretic"] carrier by means of electrophoresis.

2. In the context of a method for detecting genetic variation employing a hybridization reaction of a nucleic acid sample and a nucleic acid probe, a method for detecting genetic variation characterized in that a nucleic acid probe is fixed within an electrophoretic carrier, a nucleic acid sample is made to move within the electrophoretic carrier by means of electrophoresis, a hybridization reaction is caused to be carried out, and [a portion of] the aforesaid nucleic acid sample that has not bonded with the aforesaid nucleic acid probe is made to move by means of electrophoresis and is removed from the aforesaid electrophoretic carrier.

3. In the context of a method for detecting genetic variation employing a hybridization reaction of a nucleic acid sample and a nucleic acid probe, a method for detecting genetic variation characterized in that a nucleic acid probe is fixed within an electrophoretic carrier, a nucleic acid sample is made to move within the electrophoretic carrier by means of electrophoresis, a hybridization reaction is caused to be carried out, following which the aforesaid electrophoretic carrier is then heated and [a portion of] the aforesaid nucleic acid sample that has not bonded with the aforesaid nucleic acid probe is thereafter made to move by means of electrophoresis and is removed from the aforesaid electrophoretic carrier.

4. In the context of a method for detecting genetic variation employing a hybridization reaction of a nucleic acid sample and a nucleic acid probe, a method for detecting genetic variation characterized in that a nucleic acid probe is fixed within an electrophoretic carrier, a nucleic acid sample is made to move within the electrophoretic carrier by means of electrophoresis, a hybridization reaction is caused to be carried out, [a portion of] the aforesaid nucleic acid sample that has not bonded with the aforesaid nucleic acid probe is made to move by means of electrophoresis and is removed from the electrophoretic carrier, a labeled nucleic acid probe is furthermore made to

move within the electrophoretic carrier by means of electrophoresis, a hybridization reaction is caused to be carried out, [a portion of] the aforesaid labeled nucleic acid probe that has not bonded with the aforesaid nucleic acid probe is made to move by means of electrophoresis and is removed from the electrophoretic carrier, and the label of [a portion of] the labeled nucleic acid probe that has bonded with the aforesaid nucleic acid sample is thereafter detected.

5. In the context of a method for detecting genetic variation employing a hybridization reaction of a nucleic acid sample and a nucleic acid probe, a method for detecting genetic variation characterized in that a nucleic acid probe is fixed within an electrophoretic carrier, a nucleic acid sample is made to move within the electrophoretic carrier by means of electrophoresis, a hybridization reaction is caused to be carried out, following which the aforesaid electrophoretic carrier is heated, [a portion of] the aforesaid nucleic acid sample that has not bonded with the aforesaid nucleic acid probe is thereafter made to move by means of electrophoresis and is removed from the aforesaid electrophoretic carrier, a labeled nucleic acid probe is furthermore made to move within the electrophoretic carrier by means of electrophoresis, a hybridization reaction is caused to be carried out, following which the aforesaid electrophoretic carrier is then heated, [a portion of] the aforesaid labeled nucleic acid probe that has not bonded with the aforesaid nucleic acid probe is thereafter made to move by means of electrophoresis and is removed from the electrophoretic carrier, and the label of [a portion of] the labeled nucleic acid probe that has bonded with the aforesaid nucleic acid sample is thereafter detected.

6. A method for detecting genetic variation according to claim 4 or 5 characterized in that the label is a fluorescent substance or pigment, and these are detected within the electrophoretic carrier.

7. A method for detecting genetic variation according to claim 4 or 5 characterized in that the label is an enzyme, and a fluorescent substance or pigment produced as a result of enzymatic reaction caused by said enzyme is either detected within the electrophoretic carrier or is detected [after being] made to move out of the aforesaid electrophoretic carrier by means of electrophoresis.

8. In the context of an apparatus for detecting genetic variation employing a hybridization reaction of a nucleic acid sample and a nucleic acid probe, an apparatus for detecting genetic variation characterized in that it is equipped with an electrophoretic carrier within which is fixed a nucleic acid probe for causing hybridization of a nucleic acid sample, a DC voltage application means that applies, by way of cathode-side electrolytic solution and anode-side electrolytic solution, a DC voltage to the electrophoretic carrier within which

the aforesaid nucleic acid probe is fixed, and a measurement means that measures fluorescence or absorbance of light within the aforesaid electrophoretic carrier or within the aforesaid cathode-side electrolytic solution.

9. An apparatus for detecting genetic variation according to claim 8 characterized in that the measurement means measures fluorescence or absorbance of light within the cathode-side electrolytic solution, and in that provided therein is a membrane, allowing electrolytic solution to pass but not transmitting fluorescent substance or pigment, for concentrating the fluorescent substance or pigment that is within the cathode-side electrolytic solution and that is [to be] measured by the aforesaid measurement means.

10. An apparatus for detecting genetic variation according to claim 8 or 9 characterized in that it is equipped with a means for controlling the temperature of the aforesaid electrophoretic carrier.

11. An electrophoretic carrier within which is fixed a nucleic acid probe that is employed in a hybridization process for a nucleic acid sample and a nucleic acid probe or in a method for detecting genetic variation employing a hybridization reaction of a nucleic acid sample and a nucleic acid probe.

3. Detailed Description of Invention

Industrial Field of Application

The instant invention pertains to a hybridization process for a nucleic acid sample and a method for detecting genetic variation employing same method and an apparatus therefor, and in particular pertains to an apparatus and a method for detecting genetic variation rapidly and in a fashion facilitating automation thereof.

Conventional Art

A conventional method for detecting genetic variation employing a hybridization reaction wherein either a nucleic acid (DNA or RNA) sample or a DNA (RNA) probe (DNA (RNA) fragments possessing base sequences complementary to target DNA (RNA)) is fixed within a solid phase is described at *Proc. Natl. Acad. Sci. USA*, Vol. 80 (1983), pp. 278 - 282.

In this method, a DNA fragment sample separated by molecular weight by means of electrophoresis is first transferred onto a nitrocellulose membrane and fixed thereon, this membrane is thereafter immersed within a solution containing a DNA probe, and a hybridization reaction is carried out. During the hybridization reaction, the higher the degree of complementarity between base sequences therein the stronger will be the bonding between the DNA fragment sample and the DNA probe, dissociation thereof not

occurring even at high temperature. Now, if the DNA fragment sample possesses perfect complementarity with respect to the DNA probe it will not dissociate therefrom, but if there is no complementarity or if there is less than perfect complementarity [the DNA probe can] then be washed [off and removed] at a temperature such as will permit dissociation therefrom. If the DNA fragment sample possesses perfect complementarity with respect to the DNA probe the DNA probe will remain bonded to the membrane and will be left behind, where it can be detected; but if not, the DNA probe will be washed off the membrane and will not be detected. As described above, this method makes it possible to determine whether or not the DNA fragment sample possesses perfect complementarity with respect to the DNA probe. Accordingly, by using a DNA fragment possessing perfect complementarity with respect to a normal target gene as the DNA probe, it is possible to determine whether the target gene within a DNA fragment sample is normal or whether it is abnormal due to the presence of point mutation, insertion, deletion, or other such variation, permitting detection of genetic variation.

Problem to Be Solved by Invention

In the conventional method described above, because the hybridization reaction takes place as a result of passive diffusion between a DNA fragment sample fixed on a nitrocellulose membrane (solid phase) and a DNA probe within solution, there has been the problem that reaction rate is slow. Moreover, there has also been the problem that [the conventional method] comprises operations which do not lend themselves to automation, these being the filling and discharge of the several solutions during carrying out of reaction and during washing.

The object of the instant invention is to provide a hybridization process and a method for detecting genetic variation employing said method and an apparatus for use therein that are rapid, that lend themselves to automation, wherein hybridization reaction rate is fast, and wherein there are few operations that do not lend themselves to automation, such as filling and discharge of solutions and so forth.

Means for Solving Problem

In order to accomplish the aforesaid object, in the instant invention a DNA probe is fixed on an electrophoretic carrier, above and below which are arranged, by way of [intervening] buffer solution, two electrodes, a nucleic acid fragment sample or the like undergoes forced movement by means of electrophoresis, and hybridization reaction(s) and washing are carried out.

That is, the instant invention, in the context of a hybridization process for a nucleic acid sample and a nucleic acid probe, is a hybridization process for a nucleic acid sample characterized in that a nucleic acid probe is fixed within an electrophoretic carrier and a nucleic acid sample is made to move within the electrical [*probably a typo for* "electrophoretic"] carrier by means of electrophoresis. In this hybridization process, because the nucleic acid sample undergoes forced movement across the electrophoretic

carrier on which the DNA probe is fixed, this permits the hybridization reaction to take place more rapidly, and the reaction to be completed in a shorter time, than would be the case with the aforesaid conventional method.

Furthermore, the instant invention, in the context of a method for detecting genetic variation employing a hybridization reaction of a nucleic acid sample and a nucleic acid probe, is a method for detecting genetic variation characterized in that a nucleic acid probe is fixed within an electrophoretic carrier, a nucleic acid sample is made to move within the electrophoretic carrier by means of electrophoresis, a hybridization reaction is caused to be carried out, and [a portion of] the aforesaid nucleic acid sample that has not bonded with the aforesaid nucleic acid probe is made to move by means of electrophoresis and is removed from the aforesaid electrophoretic carrier.

The aforesaid method for detecting genetic variation may be carried out such that it employs two types of nucleic acid probes; i.e., a nucleic acid probe which is fixed on the electrophoretic carrier (the fixed probe), and a labeled second nucleic acid probe (the labeled probe), which is [used to] further hybridize [the portion of] the nucleic acid sample that has bonded to the aforesaid fixed probe. That is, this method for detecting genetic variation may be carried out such that a nucleic acid probe is fixed within an electrophoretic carrier, a nucleic acid sample is made to move within the electrophoretic carrier by means of electrophoresis, a hybridization reaction is caused to be carried out, [a portion of] the aforesaid nucleic acid sample that has not bonded with the aforesaid nucleic acid probe is made to move by means of electrophoresis and is removed from the electrophoretic carrier, a labeled nucleic acid probe is furthermore made to move within the electrophoretic carrier by means of electrophoresis, a hybridization reaction is caused to be carried out, [a portion of] the aforesaid labeled nucleic acid probe that has not bonded with the aforesaid nucleic acid probe is made to move by means of electrophoresis and is removed from the electrophoretic carrier, and the label of [a portion of] the labeled nucleic acid probe that has bonded with the aforesaid nucleic acid sample is thereafter detected.

Moreover, it is possible with any of the aforesaid methods to add an operation wherein the electrophoretic carrier is heated after causing the hybridization reaction to be carried out. It is desirable that the temperature to which [the electrophoretic carrier] is heated be such that dissociation does not occur if the nucleic acid sample possesses perfect complementarity with respect to the nucleic acid probe but such that dissociation will occur if there is no complementarity or if there is less than perfect complementarity. While this temperature will vary depending on the lengths and base sequences of the nucleic acid sample and nucleic acid probe, and depending on the genetic variation being detected; for example, when using a nucleic acid probe that is 19 bases in length to detect a point mutation within the ß-globin gene, a temperature of 55° C is preferred. Also, this heating of the electrophoretic carrier permits an increase in the precision of the method for detecting genetic variation employing a hybridization reaction of a nucleic acid sample and a nucleic acid probe.

As the label substance for the aforesaid labeled nucleic acid probe, one may employ any [suitable substance] so long as it is capable of being detected, and, while ^{32}P or another such radioisotope may be used, it is preferable to employ a fluorescent substance or pigment, or an enzyme that produces a fluorescent substance or pigment as a result of a reaction, and specifically, one may [preferably] employ, for example, fluorescein isothiacynate [*probably a typo for* "isothiocyanate"] (FITC), esterase, or the like. Also, measurement of this fluorescent substance or this pigment may be carried out either within the aforesaid electrophoretic carrier or [after causing the fluorescent substance or pigment] to move out of the aforesaid electrophoretic carrier by means of electrophoresis.

In addition, with respect to an apparatus for detecting genetic variation for the purpose of carrying out the aforesaid method for detecting genetic variation, the instant invention, in the context of an apparatus for detecting genetic variation employing a hybridization reaction of a nucleic acid sample and a nucleic acid probe, is an apparatus for detecting genetic variation characterized in that it is equipped with an electrophoretic carrier within which is fixed a nucleic acid probe for causing hybridization of a nucleic acid sample, a DC voltage application means that applies, by way of cathode-side electrolytic solution and anode-side electrolytic solution, a DC voltage to the electrophoretic carrier within which the aforesaid nucleic acid probe is fixed, and a measurement means that measures fluorescence or absorbance of light within the aforesaid electrophoretic carrier or within the aforesaid cathode-side electrolytic solution. Furthermore, this apparatus for detecting genetic variation may be such that when the measurement means measures fluorescence or absorbance of light within the cathode-side electrolytic solution, there may be provided therein a membrane, allowing electrolytic solution to pass but not transmitting fluorescent substance or pigment, for concentrating the fluorescent substance or pigment that is within the cathode-side electrolytic solution and that is [to be] measured by the aforesaid measurement means. While any [suitable membrane] may be used as this membrane so long as it provides the aforesaid function, one may employ, for example, a porous glass membrane made of quartz.

Furthermore, this apparatus for detecting genetic variation may be equipped with control means for controlling the temperature of the aforesaid electrophoretic carrier.

Moreover, the instant invention concerns an electrophoretic carrier within which is fixed a nucleic acid probe that is employed in the aforesaid hybridization process for a nucleic acid sample and a nucleic acid probe or method for detecting genetic variation employing a hybridization reaction of a nucleic acid sample and a nucleic acid probe.

Action

After adding the DNA fragment sample to the top surface of the electrophoretic carrier, a DC voltage is applied between the two electrodes, and the DNA fragment sample undergoes forced movement within the carrier. This permits the hybridization reaction to take place more rapidly than is the case when the DNA fragment sample is passively diffused.

Furthermore, [the portion of] the DNA fragment sample that did not bond, or that bonded only weakly, during the hybridization reaction is removed by means of electrophoresis. This permits attainment of a method suitable for automation, as washing operations involving filling and discharge of solutions and so forth are [no longer] required.

Moreover, measurement of fluorescence or absorbance of light from the label substance, a hybridization reaction reactant, may be carried out either [while the label is] within the aforesaid electrophoretic carrier or [while it is] within the cathode-side electrolytic solution; furthermore, if measurement is carried out [while the label is] within the latter, the cathode-side electrolytic solution, measurement sensitivity may be increased through the provision of a membrane that concentrates the fluorescent substance or pigment.

Embodiments

Below, we describe the instant invention in further detail through the use embodiments; however, the instant invention is not to be limited by these embodiments.

EMBODIMENT 1

We describe the instant embodiment with reference to Fig. 1 (a) and (b).

An electrophoretic carrier 1 within which a DNA probe [was] fixed [was] first prepared as follows. The DNA probe [was prepared] by using the phosphoamidide method, currently in wide use, to synthesize a DNA fragment (3'-GAGGACTCCTCTTCAGACG-5') that was perfectly complementary to the base sequence from the 14th to the 32nd [base] from the 5' end of the human ß-globin gene. However, at the final step of synthesis, i.e. the step of adding guanine (G) at the 5' end, we used the method of L.M. Smith et al, wherein deoxyguanosine containing an amino group at its 5' end is employed instead of deoxyguanosine, to introduce an amino group at the 5' end of the DNA fragment. After purifying this DNA probe using high-performance liquid chromatography (HPLC), we then added [the purified DNA probe] to a 2.5% aqueous solution of acrolein and allowed this to react for 30 min over an ice bath. After dialyzing this well using PBS buffer solution, we further added 5% [of] acrylamide - N,N'-methylenebisacrylamide solution (acrylamide : N,N'-methylenebisacrylamide = 20 : 1), N,N,N',N'-tetramethylethylene diamine for a final concentration of 0.08%, and ammonium persulfate for a final concentration of 0.1%, and poured this into a glass tube 2 and allowed this to gel to obtain an electrophoretic carrier 1.

As the DNA fragment sample, we used normal, unmutated human ß-globin gene ($ß^A$) and we used ß-globin gene ($ß^B$) from a patient suffering from sickle cell anemia, wherein the adenosine [*may be a typo for* "adenine"] (A) at the 20th [base] from the 5' end had mutated (point mutation) to thymine (T), which had been broken [into fragments]

using restriction enzyme BamHI (fragments approximately 1,800 base pairs in length including region in vicinity of 5' end of ß-globin gene).

After using heat to denature the aforesaid DNA fragment sample, forming single-stranded DNA, this was poured onto the top end of the electrophoretic carrier 1, on which the DNA probe had been fixed and which was being maintained at 45° C by means of a temperature controller 3, and a DC power supply 10 was used to apply a voltage between an anode 6, present within an upper electrolytic solution tank 4, and a cathode 9, present within a lower electrolytic solution tank 7. Because this causes the DNA fragment sample to undergo forced [movement] into the electrophoretic carrier 1 by means of electrophoresis, the hybridization reaction can proceed more rapidly than would be the case with no electrophoresis, when [the DNA fragment sample] is passively diffused.

Then, after using the temperature controller 3 to change the temperature of the electrophoretic carrier 1 to 55° C, a voltage was again applied between the two electrodes 6,9, and the [portion of the] DNA fragment sample that was dissociated because of lack of perfect complementarity with respect to the DNA probe was removed by means of electrophoresis.

Furthermore, after returning the temperature of the electrophoretic carrier to 45° C, a second DNA probe, which had been labeled with esterase, was poured onto the top end of the electrophoretic carrier 1, and electrophoresis was carried out. This DNA probe (the labeled probe) was a DNA fragment (3'-CCACTTGCACCTACTTCAAC-5') synthesized using the phosphoamidide method in the same manner as the probe fixed on the electrophoretic carrier 1 (the fixed probe), the 5' end thereof being labeled with esterase, but complementary with respect to a different region of the ß-globin gene than the fixed probe; to wit, to the base sequence from the 53rd to the 72nd [base] from the 5' end thereof. Accordingly, if the DNA fragment sample bonds to the fixed probe and remains within the electrophoretic carrier 1, the labeled probe will bond to a different region of the DNA fragment sample and will likewise remain within the electrophoretic carrier 1; however, if the DNA fragment sample does not remain [within the electrophoretic carrier 1], the labeled probe will not remain within the electrophoretic carrier 1 but will pass therethrough.

Finally, FDA (fluorescein diacetate), which acts as substrate for the labeled esterase enzyme, was likewise poured onto the top end of the electrophoretic carrier 1, electrophoresis was carried out, and fluorescence of fluorescein, the fluorescent substance produced by the enzymatic reaction, was thereafter measured within the electrophoretic carrier 1.

Light exiting from a xenon lamp light source 11 was made to pass through an interference filter 12, light of wavelength 490 nm being selected, following which this was condensed by a lens 13 and the electrophoretic carrier 1 was irradiated with excitation light. From a direction that was 90° with respect to the excitation light, [after] passing through a lens 17, a cutoff filter 18, and an interference filter 19, light of wavelength in the

vicinity of 510 nm was selectively detected at a photomultiplier 20. Moreover, a window 16 was provided at the side opposite an incident[-side] window 14, and the effect of scattered light was reduced by guiding to the outside [some of the] excitation light that had passed through the electrophoretic carrier 1. The output from the photomultiplier 20 was amplified at an amplifier 21, and this was thereafter recorded on a recorder 22.

As a result of measurement, [it was found that] with the DNA fragment sample containing normal, unmutated human ß-globin gene ($ß^A$) fragments and for which there was perfect complementarity with respect to the fixed DNA probe, fluorescence was detected; but with the DNA fragment sample containing ß-globin gene ($ß^B$) fragments from a patient suffering from sickle cell anemia, in which there was a mutation (point mutation) and for which complementarity with respect to the fixed DNA probe was lacking only at a single base, fluorescence was not detected. In order to confirm [this result], we replaced the fixed DNA probe with a [fragment] possessing perfect complementarity (3'-GAGGACACCTCTTCAGACG-5') with respect to the $ß^B$ gene and carried out measurements in the same fashion [as before], upon which [it was found that] fluorescence was not detected for the DNA fragment sample containing $ß^A$ gene fragments, but [fluorescence] was detected for the [DNA fragment sample containing] $ß^B$ gene fragments. Because it was possible to distinguish between gene fragments containing variation and gene fragments not containing variation based on whether or not fluorescence was detected, we were thus able to detect variation (point mutation) present within the ß-globin gene fragments.

Moreover, whereas in the instant embodiment we employed an enzyme (esterase) as the label substance and measured fluorescence of FDA produced as a result of enzymatic reaction, one may also employ FITC or other such fluorescent substance as label substance and measure the fluorescence thereof directly without employment of an enzyme or enzymatic reaction.

As described above, the instant embodiment permits attainment of an apparatus and method for detecting genetic variation rapidly and in a fashion facilitating automation thereof.

EMBODIMENT 2

Next, we describe a second embodiment with reference to Fig. 2.

The difference between the instant embodiment and Embodiment 1 is that fluorescence of the fluorescein fluorescent substance [was] measured not within the electrophoretic carrier 1 but within the lower electrolytic solution 8. After causing the FDA to move into the electrophoretic carrier 1 by means of electrophoresis at the last step of the above embodiment, electrophoresis was again continued, causing the fluorescein fluorescent substance produced as a result of enzymatic reaction to migrate into the lower electrolytic solution 8. In addition, fluorescence of fluorescein within the lower electrolytic solution was measured using the apparatus shown at Fig. 2.

In addition to benefits similar to those of the above embodiment, because fluorescence of fluorescein is measured not within the electrophoretic carrier, which displays much scattering of light and interfering fluorescence, but within the electrolytic solution, which displays little of these, the instant embodiment possesses the benefit that it allows fluorescence to be measured with high sensitivity.

EMBODIMENT 3

Next, we describe a third embodiment with reference to Fig. 3.

The difference between the instant embodiment and Embodiment 2 is the fact that a small-volume electrolytic solution tank 25 is constituted as a result of arrangement of a porous glass membrane 24 attached to a membrane retaining fixture 23 made of acrylic between the bottom end of the electrophoretic carrier 1 and the lower electrolytic solution 8. The aforesaid porous glass membrane 24 is quartz glass that, having been reacted with tetramethoxysilane in a solvent containing methanol and water according to the sol-gel method, possesses properties such that it allows the electrolyte(s) of the electrolytic solution to be transmitted [i.e., to pass] but does not allow the fluorescent substance to be transmitted [i.e., to pass]. Accordingly, FDA fluorescent substance produced as a result of enzymatic reaction will be concentrated within the small-volume electrolytic solution tank 25. In the instant embodiment, a pipette 27 was used to guide electrolytic solution containing fluorescent substance concentrated as a result of the above process through a guide hole 26 and into a fluorescence cell 28. The pipette 27 was retained by a mechanism 29 [capable of] rotary and vertical [movement]. Fluorescence of the fluorescent substance within the fluorescence cell 28 was measured using an optical system similar to that shown in Fig. 2.

In addition to benefits similar to those of Embodiment 2, because the instant invention permits the FDA fluorescent substance to be concentrated within a small volume of electrolytic solution, it possesses the benefit that it allows fluorescence to be measured with even higher sensitivity.

Benefit of Invention

In the instant invention, because the DNA fragment sample undergoes forced movement within the electrophoretic carrier by means of electrophoresis, this permits the hybridization reaction to take place more rapidly, and the reaction to be completed in a shorter time, than would be the case were it to undergo passive diffusion as in the conventional method employing a nitrocellulose membrane. Furthermore, [the instant invention permits] easy removal, by means of electrophoresis, without employment of washing operations involving filling and discharge of solutions and so forth, of [the portion of] the DNA sample that does not bond, or that bonds only weakly, during the hybridization reaction. Accordingly, [the instant invention] permits attainment of a method for detecting genetic variation rapidly and in a fashion facilitating automation thereof.

Moreover, the instant invention permits an increase in measurement sensitivity as a result of concentration of the fluorescent substance or pigment [used as] label substance.

4. Brief Description of Drawings

Fig. 1 (a) and (b) are, respectively, a longitudinal cross-section and a lateral cross-section of an apparatus used in a first embodiment of the instant invention, Fig. 2 is a longitudinal cross-section of an apparatus used in a second embodiment of the instant invention, and Fig. 3 is an enlarged view of a portion of a longitudinal cross-section of an apparatus used in a third embodiment of the instant invention.

1...electrophoretic carrier; 2...glass tube; 3...temperature controller; 4...upper electrolytic solution tank; 5...upper (anode-side) electrolytic solution; 6...anode; 7...lower (cathode-side) electrolytic solution tank; 8...lower (cathode-side) electrolytic solution; 9...cathode; 10...DC power supply; 11...light source; 12,19...interference filter; 13,17...lens; 14...incident[-side] window; 15...detection window; 16...window; 18...cutoff filter; 20...photomultiplier; 21...amplifier; 22...recorder; 23...membrane retaining fixture; 24...porous glass membrane; 25...small-volume electrolytic solution tank; 26...guide hole; 27...pipette; 28...fluorescence cell; 29...mechanism [capable of] rotary and vertical [movement].

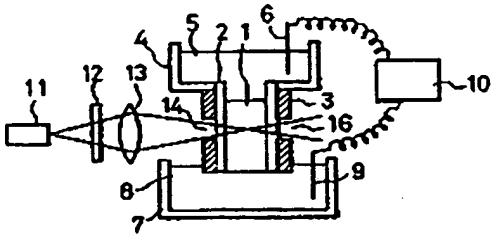
Fig. 1

Fig. 2

Fig. 3

第 1 図

(a)



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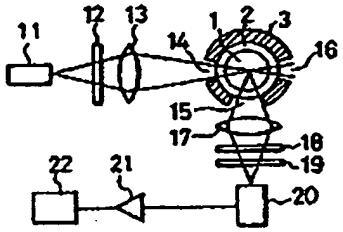
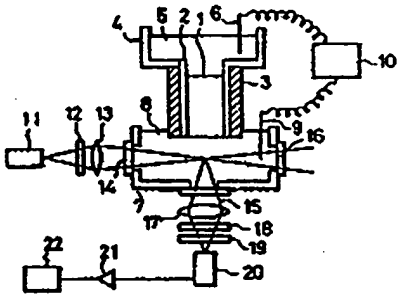


第 2 図



第 3 図